(ii) a second antibody which is capable of specifically binding to a second binding site on the antigen, wherein the second antibody is free,

thereby forming, when the antigen is present in the sample, an agglutinate comprising the first antibody, the antigen, and the second antibody; followed by

(b) optically measuring the amount of the agglutinate formed in (a),

wherein one of the antibodies has high specificity for the antigen while the other antibody does not have strict specificity for the antigen.

8. The immunoassay of Claim 7, wherein said optically measuring comprises spectrophotometrically measuring decreasing light transmission due to formation of the agglutinate.

9. The immunoassay according to Claim 7, wherein the amount of the antigen in the sample is determined from a calibration curve.

10. The immunoassay of Claim 7, wherein the sample contains an undetectable amount of the antigen.

11. The immunoassay of Claim 7, wherein the sample contains a detectable quantity of the antigen.

12. The immunoassay of Claim 7, wherein the insoluble carrier is selected from the group consisting of organic polymeric substances, inorganic substances, cell membranes, hemocytes and microorganisms.

13. The immunoassay according to Claim 7, wherein the insoluble carrier is a latex particle.

14. The immunoassay of Claim 7, wherein the insoluble carrier is silica or alumina.

15. The immunoassay of Claim 7, wherein the insoluble carrier has an average particle size of 0.05 to 1 μm.

16. The immunoassay of Claim 7, wherein the sample is a buffered aqueous solution.

17. The immunoassay of Claim 7, wherein the antigen is selected from the group consisting of insulin, HCG-β, growth hormone, TSH, LH, FSH, prolactin, thyroxin, triiodothyronine, gastrin, glucagon, somatostatin, enzymes, serum proteins, clotting-fibrinolytic factors, $HbA_1C$, tumor-associated antigens, DNA-binding protein factors, cytokines, bacteria, viruses, and protozoa.

18. The immunoassay of Claim 7, wherein the sample does not contain an immune reaction-accelerating component.

19. The immunoassay of Claim 18, wherein the immune reaction-accelerating component is polyethylene glycol 6000.

20. The immunoassay of Claim 18, wherein the first antibody is a monoclonal antibody and the second antibody is a polyclonal antibody.

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21. (Amended) An agglutination immunoassay for detecting an antigen in a sample, comprising:

(a) sequentially contacting the sample with

(i) a first antibody which is capable of specifically binding to a first binding site on the antigen, wherein the first antibody is free, and then

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(ii) a second antibody which is capable of specifically binding to a second binding site on the antigen, wherein the second antibody is immobilized on an insoluble carrier,

thereby forming, when the antigen is present in the sample, an agglutinate comprising the first antibody, the antigen, and the second antibody; followed by

(b) optically measuring the amount of the agglutinate formed in (a).

wherein one of the antibodies has high specificity for the antigen while the other antibody does not have strict specificity for the antigen.

22. The immunoassay of Claim 21, wherein said optically measuring comprises spectrophotometrically measuring decreasing light transmission due to formation of the agglutinate.

23. The immunoassay according to Claim 21, wherein the amount of the antigen in the sample is determined from a calibration curve.

24. The immunoassay of Claim 21, wherein the sample contains an undetectable amount of the antigen.

25. The immunoassay of Claim 21, wherein the sample contains a detectable quantity of the antigen.

26. The immunoassay of Claim 21, wherein the insoluble carrier is selected from the group consisting of organic polymeric substances, inorganic substances, cell membranes, hemocytes and microorganisms.

27. The immunoassay according to Claim 21, wherein the insoluble carrier is a latex particle.

28. The immunoassay of Claim 21, wherein the insoluble carrier is silica or alumina.

29. The immunoassay of Claim 21, wherein the insoluble carrier has an average particle size of 0.05 to 1 μm.

30. The immunoassay of Claim 21, wherein the sample is a buffered aqueous solution.

31. The immunoassay of Claim 21, wherein the antigen is selected from the group consisting of insulin, HCG-β, growth hormone, TSH, LH, FSH, prolactin, thyroxin, triiodothyronine, gastrin, glucagon, somatostatin, enzymes, serum proteins, clotting-

fibrinolytic factors, $HbA_1C$, tumor-associated antigens, DNA-binding protein factors, cytokines, bacteria, viruses, and protozoa.

32. The immunoassay of Claim 21, wherein the sample does not contain an immune reaction-accelerating component.

33. The immunoassay of Claim 32, wherein the immune reaction-accelerating component is polyethylene glycol 6000.

34. The immnuoassay of Claim 21, wherein the first antibody is a monoclonal antibody and the second antibody is a polyclonal antibody.--

SUPPORT FOR THE AMENDMENTS

The amendments to Claims 7 and 21 are supported by the specification at page 5, lines 12-19. No new matter is believed to have been added to this application by these amendments.

REMARKS

Claims 7-34 remain active in the present application.

The present invention relates to an immunoassay for detecting an antigen in a sample. An important feature of the present method is that two antibodies are used to bind the antigen, and each antibody is contacted with the sample sequentially to form an agglutinate comprising the antigen and the two antibodies (see (i) and (ii) in Claims 7 and 21). An important feature of the claimed method is that one of the antibodies has high specificity for the antigen while the other antibody does not have strict specificity for the antigen (see the last two lines of Claims 7 and 21). The present inventors have discovered that this two-step antibody binding reaction provides a assay method having high sensitivity and low cost.